Remarks

The August 21, 2003 Official Action has been carefully reviewed. In view of the amendments submitted herewith and these remarks, favorable reconsideration and allowance of this application are respectfully requested.

At the outset it is noted that a shortened statutory response period of three (3) months was set forth in the August 21, 2003 Official Action. The initial due date for response, therefore, was November 21, 2003. A petition for a 1 month extension of the response period is presented with this response, which is being filed within the one month extension period.

At page 2 of the Official Action, the Examiner has maintained the objection to the drawings for allegedly being informal. The Examiner has indicated that this objection may no longer be held in abeyance.

The Examiner has also objected to the specification for containing an embedded figure on page 89 and for containing a large gap at page 94.

At page 12 of the Official Action, the Examiner has objected to claim 27 for improper capitalization, claims 44 and 68 for allegedly claiming the same subject matter as claim 2, claim 48 for allegedly containing redundant language, and claims 61 and 68 for containing misspelled words.

The Examiner has also maintained the rejection of claim 1 under 35 U.S.C. §102(e) as alleged anticipation by US Patent No. 5,962,290.

Claims 2, 25-27, 31-37, 39, 44, 47-49, 51, 52, 55-60, and 64-68 are rejected under 35 U.S.C. §103(a). Specifically, claims 2, 25, 26, 31-37, 39, 44, 47-49, 55-60, and 64-68 are allegedly unpatentable over U.S. Patent No. 5,962,290 in view of MacNeil et al. (Ann. NY Acad. Sci (1994) 721:123-132). The Examiner also contends that claim 27 is allegedly unpatentable over U.S. Patent No. 5,962,290 in view of MacNeil et al. and Kao et al. (Science (1994) 265:509-512).

Additionally, claims 51 and 52 are allegedly unpatentable over U.S. Patent No. 5,962,290 in view of MacNeil et al. and U.S. Patent No. 5,190,871.

At pages 13 through 15 of the Official Action, the Examiner has rejected claims 26, 54, 59, and 61-63 under 35 U.S.C. §112, second paragraph as allegedly being indefinite.

The Examiner has also rejected claims 25-27, 31-37, 39, 44, and 47-68 under 35 U.S.C. §112, first paragraph as allegedly containing new matter. The Examiner's position in support of this rejection is found at pages 15 through 17 of the Official Action.

The Examiner has additionally rejected claims 25-27, 31-37, 39, 44, 47-51, 53-60, and 64-68 for allegedly failing to satisfy the written description requirement under 35 U.S.C. §112, first paragraph. The Examiner's position in support of this rejection can be found at pages 17 through 20 of the Official Action.

At pages 20 through 22 of the Official Action, the Examiner has also rejected claims 39, 57, 59, 60, 64, and 65 for alleged non-compliance with the enablement requirement under 35 U.S.C. §112, first paragraph.

The foregoing objections and rejections constitute all of the grounds set forth in the August 21, 2003 Official Action for refusing the present application.

Amended figures are provided herewith which are believed to overcome the objections set forth by the Draftsperson in the September 11, 2001 Official Action. Notably, Figure 10A was divided into new Figures 10A and 10B in order to meet the Draftsperson requirements. Accordingly, original Figure 10B was renamed 10C. Figures 12 and 14 were also divided into Figures 12A and 12B and Figures 14A, 14B, 14C and 14D, respectively. No new matter has been added by the amendments to the figures. Therefore, Applicants respectfully request the withdrawal of this objection.

The specification has been amended to delete the

large gap on page 94. The specification has also been amended to remove the embedded figure on page 89 and add the removed figure to the formal drawings of the application, as suggested by the Examiner. Accordingly, the specification has been amended to reference the newly added figure.

No new matter has been added to the specification by these amendments. These amendments are believed to overcome the above-noted specification objections and Applicants respectfully request their withdrawal.

Certain of the present claim amendments are believed to overcome the objections to the claims set forth in the August 21, 2003 Official Action. Specifically, claim 27 has been amended to capitalize Streptomyces as requested by the Examiner. Typographical errors were also corrected in claims 61 and 68 per the Examiner's request. The redundant language perceived by the Examiner in claim 48 has also been removed by amendment. Lastly, the Examiner objected to claims 44 and 68 under 37 C.F.R. §1.75 as being substantial duplicates of claim 2. The present amendment cancels claim 44. Applicants contend claim 68 differs from claim 2 because the first nucleic acid portion of claim 2 encodes at least a loading module whereas the first nucleic acid portion of claim 68, due to the amendments to claim 67, may encode any multiple domains from a Type I PKS. Accordingly, Applicants submit the claims are plainly of different scope and respectfully request the withdrawal of the above-mentioned objections to the claims.

New claims 69 and 70 are presented with this amendment and their entry is respectfully requested. Claims 26, 44, and 59 have been cancelled.

New claim 69 depends from amended claim 67. Claim 69 recites that the first portion of the hybrid polyketide synthase (PKS) comprises a loading module. Support for this recitation can be found throughout the specification, for example, at page 50, lines 18-21.

New claim 70 depends from newly added claim 69.

Claim 70 recites the loading module of the hybrid PKS gene is selected from rapamycin, FK506, and ascomycin -producing polyketide synthases. Support for this recitation can be found at page 16, lines 3-7.

No new matter has been introduced into this application by reason of any of the claim amendments presented herewith. For the most part, the effect of the foregoing amendments is merely to make express that which was implicit in the application as originally presented.

CLAIMS 26, 54, 59, AND 61-63, AS AMENDED, MEET THE REQUIREMENTS OF 35 U.S.C. §112, SECOND PARAGRAPH

The Examiner has rejected claims 26, 54, 59, and 61-63 under 35 U.S.C. §112, second paragraph, as certain claim terms are considered indefinite.

Specifically, the Examiner has rejected claim 26 for recitation of the term "multiplicity." Inasmuch as claim 26 has been canceled, this rejection has been rendered moot.

The Examiner has also rejected claim 54 for the recitation of "enzyme from the rapamycin system which ... effects connection of the polyketide chain to an amino acid chain." The Examiner contends in this regard that the nature of the "enzyme" is unclear from the specification and the prior art and that it is unclear if the nucleic acid encoding the "enzyme" would be included within the first or second nucleic acid portion. Applicants respectfully submit that Schwecke et al. (Proc. Natl. Acad. sci. (1995) 92:7839-7843), which was published in August 1995 and thus prior to the instant application, clearly indicate that it is the *rapP* gene which is responsible for the cyclization of rapamycin and linkage of the polyketide chain to the "amino group of an enzyme-bound pipecolyl moiety" (see page 7841, second column, second paragraph). Additionally, Applicants have amended claim 54 to indicate that the rapamycin segment replaces the region which encodes for the thioesterase. Inasmuch as claim

54 indicates that the thioesterase is included in the first Type 1 PKS which is, according to claim 67, encoded for by the first nucleic acid portion, Applicants submit that it is clear that the rapamycin segment is included within the first nucleic acid portion.

Claim 59 has also been rejected for recitation of the term "Streptomyces tsukubaensis." It is the Examiner's contention that such a species does not exist. Applicants submit herewith Tanaka et al. (J. Amer. Chem . Soc. (1987) 109:5031-5033) wherein at page 5031, right column the species Streptomyces tsukubaensis is disclosed. Accordingly, Applicants respectfully submit that the Examiner's rejection of claim 59 for indefiniteness is untenable.

Additionally, the Examiner has rejected claim 61 for recitation of "gene comprising a plurality of modules." Specifically, the Examiner contends that genes encode proteins which contain modules, but genes do not have modules. In order to clarify the ambiguity perceived by the Examiner, Applicants have amended claim 61 to further recite a PKS gene "encoding a polyketide synthase" comprising a plurality of modules. Additionally, the Examiner contends that the claim is also ambiguous for recitation of the term "combinatorial module," particularly in reference to first and second points. Applicants respectfully submit, however, that a combinatorial module is clearly defined as "any group of contiguous domains (and domain parts), extending from a first point in a first natural module, to a second equivalent point in a second natural module" (see page 2, lines 9-13). Inasmuch as the term "combinatorial" requires the module to extend between equivalent points in natural modules, Applicants submit it is clear as to how the term "combinatorial module" further limits the hybrid polyketide synthase gene.

It is also the Examiner's position that claim 62 is indefinite for recitation of the term "adapted." In order to eliminate the ambiguity perceived by the Examiner, Applicants

have deleted the term from the claim, thereby rendering moot the Examiner's rejection of claim 62 for indefiniteness.

Lastly, the Examiner contends claim 63 is indefinite because it is allegedly unclear which KS domain is referred to in the recitation of "the KS domain of the extension module which is homologous to said loading module." In an effort to advance prosecution of the instant application, Applicants have amended claim 62 to replace the recitation in question with "adjacent KS1 domain" to clarify which KS domain is referenced in the claim.

In light of the foregoing remarks and amendments, Applicants respectfully request the rejection of claims 26, 54, 59, and 61-63 under 35 U.S.C. §112, second paragraph be withdrawn.

CLAIMS 25-27, 31-37, 39, 44, 47-68, AS AMENDED, FULLY COMPLY WITH THE REQUIREMENTS OF 35 U.S.C. §112, FIRST PARAGRAPH

The Examiner has also rejected claims 25-27, 31-37, 39, 44, and 47-68 under 35 U.S.C. §112, first paragraph as allegedly containing new matter. The Examiner has additionally rejected claims 25-27, 31-37, 39, 44, 47-51, 53-60, and 64-68 for allegedly failing to satisfy the written description requirement under 35 U.S.C. §112, first paragraph. Lastly, the Examiner has rejected claims 39, 57, 59, 60, 64, and 65 for allegedly failing to satisfy the enablement requirement under 35 U.S.C. §112, first paragraph.

As to the new matter rejections, the Examiner contends the method claimed in claim 52 lacks support in the specification. Specifically, the Examiner alleges that while the specification provides support for the claimed method employing at least one "module," the specification lacks support for the recitation of at least one "domain." In an effort to advance prosecution of the instant application, Applicants have amended claim 52 to recite "module" instead of "domain," thereby overcoming the Examiner's rejection.

It is also the Examiner's position that claim 61-63 lack support in the specification. The Examiner is mistaken in this regard as support for the subject matter of claim 61 can be found at page 9, lines 20-29. In light of the explanation of the "combinatorial modules" set forth above and the citation at page 9, lines 20-29 describing the employment of combinatorial modules in place of natural modules, Applicants submit that the invention claimed in claim 61 is adequately described in the specification. Further, claim 62 calls for a hybrid PKS gene wherein an extension module is replaced with an extension module from another PKS gene. Support for this claimed subject matter can be found broadly at page 14, lines 5-10 and exemplified in Examples 29-31. Lastly, claim 63 describes a hybrid PKS gene comprising a loading module and KS1 domain from one PKS gene and a partial extension module lacking a KS domain from a second PKS gene. Support for this claimed subject matter can be found at page 9, lines 5-19 and exemplified in Example 32. Thus, Applicants submit that there is adequate support in the specification for the claimed subject matter of claims 61-63.

Furthermore, the Examiner contends that claim 67 and dependent claims 25-27, 31-37, 39, 44, 47-51, 53-60, 64-66, and 68 contain new matter by reciting the phrase "a loading module lacking a ketosynthase (KS) activity." Accordingly, Applicants have amended claim 67 by deleting the phrase in question, thereby rendering the rejection of claim 67 moot. Applicants have, however, added new claim 69 which is dependent on claim 67 and recites that the first nucleic acid portion comprises at least a loading module lacking a ketosynthase (KS) "domain." Support for reciting that a loading module lacks a ketosynthase "domain" can be found throughout the specification, including at page 6, lines 22-23.

Turning to the written description rejections, the Examiner contends that the genus of non-specific loading

modules claimed in claim 26 are not adequately described in the specification by the single example of employing the avermectin PKS loading module. As noted hereinabove, claim 26 has been canceled thereby rendering this rejection moot. However, Applicants have added new claim 70 which specifically recites that the loading module be selected from loading modules of rapamycin, FK506 and ascomycin -producing polyketide synthases. Support for this recitation can be found at page 16, lines 3-7.

The Examiner also rejects claim 67 and dependent claims 25-27, 31-37, 39, 44, 47-51, 53-60, 64-66, and 68 for allegedly failing to satisfy the written description requirement of 35 U.S.C. §112, first paragraph. Specifically, the Examiner contends that the recitation of a loading module which lacks KS activity would include loading modules which contain an inactive KS domain. The Examiner alleges that the specification only describes loading modules consisting of acyltransferase and acyl carrier protein domains and, therefore, fails to describe loading modules comprising an inactive KS domain. As noted hereinabove, Applicants have deleted the reference to KS activity in claim 67 and have taken care to recite a loading module lacking a KS "domain" in newly added claim 69. Therefore, Applicants submit that the written description rejection of claim 67 is overcome.

As to the enablement rejection under 35 U.S.C. §112, first paragraph, the Examiner has rejected claims 39, 57, 59, 60, 64, and 65 because the specification, while enabling for making polyketides in microorganisms that naturally produce polyketides, allegedly fails to enable a skilled artisan to practice the claimed invention in microorganisms that do not naturally produce polyketides. Applicants take exception to the Examiner's position in this regard. In the interest of advancing the prosecution of the instant application, however, Applicants have amended claims 39, 57, 58, and 64 to recite the group of microorganisms provided in claim 59 and amended

claim 60 to specifically recite the Streptomyces from the group of microorganisms. In view of this amendment, claim 59 has been canceled.

Additionally, the Examiner rejected claim 59 for lack of enablement for the specific inclusion of Streptomyces tsukubaensis which the Examiner alleged was a species of unknown polyketide-producing abilities. As noted hereinabove, Tanaka et al. demonstrates the existence of the species and its ability to produce polyketides (see page 5031, right column of Tanaka et al.). Therefore, Applicants submit that the specification is fully enabling for the use of Streptomyces tsukubaensis in making polyketides.

In light of the foregoing remarks and amendments, Applicants respectfully request the withdrawal of the rejection of claims 25-27, 31-37, 39, 44, 47-68 under 35 U.S.C. §112, first paragraph.

U.S. PATENT NO. 5,962,290 FAILS TO ANTICIPATE CLAIM 1

The Examiner has maintained the rejection of claim 1 under 35 U.S.C. §102(e) as allegedly anticipated by U.S. Patent No. 5,962,290. Applicants continue to disagree with the Examiner's position.

In support of the §102(e) rejection, the Examiner cites column 10, lines 23-29 of U.S. Patent No. 5,962,290, which states that a PKS gene cluster "may be hybrid in nature with, e.g., a gene derived from a cluster for synthesis of a particular polyketide replaced with a corresponding gene from a cluster of another polyketide." It is the Examiner's position that it is "clearly the intent" of this disclosure to include Type I-Type I hybrid PKSs. Applicants disagree and submit that, based on the examples provided, the statement is in all likelihood referring to only Type II-Type II hybrid PKSs. The only thing that is clear from this disclosure is that it is ambiguous at best. However, it has long been recognized that an ambiguous reference will not support an

anticipation rejection. In re Hughes, 145 U.S.P.Q. 467 (1965). Thus, the §102 rejection based on the citation at column 10, lines 23-29 of U.S. Patent No. 5,962,290 is untenable.

Furthermore, even if the general statement at column 10, lines 23-29 were to refer to Type I-Type I hybrids, the citation is insufficient to identically disclose every limitation of claim 1 of the instant application. Indeed, the citation in U.S. Patent No. 5,962,290 clearly discusses switching entire **genes**, but claim 1 of the instant application specifically recites PKSs that contain "at least one **domain**" from a heterologous PKS.

In response to Applicants' argument that, at the priority date of U.S. Patent No. 5,962,290, only one Type I PKS gene cluster (erythromycin) was sequenced, the Examiner cites MacNeil et al. (Ann. NY Acad. Sci (1994) 721:123-132) as providing the sequence of another Type I PKS. As noted hereinbelow, Applicants submit that MacNeil et al. performed only a "limited DNA sequencing strategy" (page 125, lines 10-12) to sequence the avermectin PKS and that "obvious ambiguities" in the sequence remain (page 126, legend of Figure 2). Additionally, the alleged sequence of the avermectin PKS had not been deposited into a publicly available database to allow a skilled artisan to utilize the sequences to create hybrid PKSs. Therefore, Applicants submit that a skilled artisan would still only effectively have access to one Type I PKS gene cluster, at the time of the priority date of U.S. Patent No. 5,962,290, as a partial sequence of the avermectin PKS that was not made available to the public would be of no practical use. In light of the fact that no Type I-Type I hybrids as claimed here are disclosed in U.S. Patent No. 5,962,290 and that only one Type I PKS gene cluster had been sequenced at the time, U.S. Patent No. 5,962,290 cannot reasonably be interpreted as disclosing a PKS derived from two different Type I PKSs.

For the foregoing reasons, Applicants submit that U.S. Patent No. 5,962,290 does not teach each and every aspect of the present invention and therefore request the withdrawal of the rejection of claim 1 under 35 U.S.C. §102(e).

THE COMBINED DISCLOSURE OF U.S. PATENT NO. 5,692,290 AND MACNEIL ET AL. FAIL TO RENDER OBVIOUS CLAIMS 2, 25, 26, 31-37, 39, 44, 47-49, 55-60, AND 64-68

The Examiner has rejected claims 2, 25, 26, 31-37, 39, 44, 47-49, 55-60, and 64-68 under 35 U.S.C. §103(a) as allegedly obvious over U.S. Patent No. 5,692,290 in view of MacNeil et al. (Ann. NY Acad. Sci (1994) 721:123-132). The Examiner interprets MacNeil et al. as describing a polyketide synthase in which the first acyltransferase and acyl carrier protein domain constitute a loading module. The Examiner also contends that U.S. Patent No. 5,692,290 teaches hybrid type I-type I PKS gene clusters and that it would have been obvious to a skilled artisan to generate a hybrid PKS with a different loading module to generate novel polyketides.

Applicants continue to strenuously dispute this ground of rejection. The criterion for determining obviousness under §103 is whether the prior art supplies some motivation or incentive to one of ordinary skill in the art to arrive at the invention as claimed. In re Dow Chemical Company, 5 U.S.P.Q. 2d 1929 (Fed. Cir. 1988). Obviousness cannot be established by combining teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. In re Fine, 5 U.S.P.Q.2d (Fed. Cir. 1988). Moreover, the teaching or suggestion supporting the desirability or the combination must be found in the prior art, not in applicant's disclosure. In re Fritch, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992). Under these standards, neither of the cited references, considered singly or in combination, render obvious the invention as claimed in claims 2, 25, 26, 31-37, 39, 44, 47-49, 55-60, and 64-68.

At the outset, Applicants take exception to the Examiner's dismissal of the declarations by Drs. Simpson and Knowles as not indicative of what "**all** scientists at the time of the invention were thinking." However, their CVs show indisputably that Drs. Simpson and Knowles are experts in the field of the invention, and were so recognized at the time of the priority date of the instant application. The averments of both Dr. Simpson and Dr. Knowles that it would not have been obvious to extrapolate the Type II methods of generating hybrid PKS gene clusters to Type I systems, should warrant significantly more weight than accorded by the Examiner. Indeed, the MPEP, at §716.01(a), sets forth that "declarations containing evidence of criticality or unexpected results,..., failure of others, skepticism of experts, etc. must be considered by the examiner in determining the issue of obviousness." Clearly, the declarations of Drs. Simpson and Knowles provide evidence of the "skepticism of experts."

Additional evidence of non-obviousness is provided by the fact that more than a year after the priority date of U.S. Patent No. 5,692,290, the same inventors were still not convinced that the techniques used with Type II polyketide synthases (PKSs) to generate hybrid PKSs could be employed successfully with Type I PKSs. Indeed, at page 509, right column of Kao et al. (Science (1994) 265:509-512; cited by the Examiner), the authors (which includes Khosla) note that the "extrapolation of the same approach to study modular PKSs poses several significant conceptual and technical challenges." Therefore, even the inventors named in U.S. Patent No. 5,692,290 had no reasonable expectation that the methods of generating hybrid PKSs from Type II PKSs could successfully be used to generate Type I PKSs.

It is also noteworthy in this regard that over 3 years elapsed between the purported description of making Type I hybrids in U.S. Patent No. 5,692,290 and the first actual generation of Type I hybrids by the present inventors (see

Oliynyk et al., Chemistry and Biology (1996) 3:833-839). Applicants submit, therefore, that if, as the Examiner asserts, a skilled artisan would have had the reasonable expectation of success of employing the Type II PKS methods on Type I PKSs and had the obvious motivation to create novel polyketides as therapeutically effective antibiotics, then it should not have taken such a long time to generate the first Type I hybrids. Indeed, Khosla failed to report his first actual generation of a Type I hybrid until 1997 (see McDaniel et al., Chemistry and Biology (1997) 4:667-674). Applicants submit that this "failure of others" to generate hybrid Type I PKSs further indicates that the creation of these hybrids was non-obvious.

The Examiner further asserts in support of the obviousness rejection that U.S. Patent No. 5,692,290 teaches, at column 10, lines 23-27, hybrid PKSs. Applicants maintain that such hybrids were not Type I hybrids but rather Type II hybrids and that such hybrids, in fact, only contemplate assembling whole genes into hybrid PKSs (see claim 10; column 4, lines 43-67; column 10, lines 22-33, and column 15, lines 4-7). It is evident from the overall disclosure of U.S. Patent No. 5,692,290 that it fails to teach or suggest a hybrid PKS wherein the fragments in the PKS that are replaced are smaller than genes or where a segment comprising a portion of one module and a portion of another (i.e. a combinatorial module) is used to create a heterologous hybrid. Therefore, U.S. Patent No. 5,692,290 can not be considered to render the claimed invention obvious.

The Examiner has also asserted that U.S. Patent No. 5,692,290 teaches, at column 20, lines 45-55, a method for making a polyketide using the hybrid Type I PKS genes. Applicants submit, however, that the example referred to by the Examiner only demonstrates how a PKS can be expressed in a heterologous host to produce the natural polyketide of the PKS. U.S. Patent No. 5,692,290 fails to provide any

instruction on the production of either a heterologous or homologous hybrid Type I PKS. Importantly, Kao et al. teach that required substrates for the production of modular PKSs may not be present in a heterologous host and that "proper folding, assembly, and postranslational modification of very large foreign polypeptides are not guaranteed" (page 509, right column). The failure of U.S. Patent No. 5,692,290 to provide specific method steps or examples for the production of hybrid polyketides, considered in light of the comments in Kao et al. would prevent a skilled artisan from having the required expectation of success in attempting the actual production of hybrid polyketides.

Turning to the MacNeil et al. reference, the Examiner contends that "MacNeil et al. consider the first AT and ACP domains of both the erythromycin PKS and avermectin PKS as loading domains." Applicants, for clarification, point out that MacNeil et al. only teach that the "initial AT and ACP" of the "first synthase unit" could function to load the starter acyl group (page 125, first ful paragraph). Notably, MacNeil et al. fail to teach that the two domains encompass a separate loading module, as recognized by the instant inventors, as opposed to the two domains being components of the first module.

Additionally, the Examiner states that "MacNeil et al. provide the 'corresponding genes' suggested to be combined" by U.S. Patent No. 5,692,290. Applicants submit, however, that MacNeil et al. teach only a "limited DNA sequencing strategy" (page 125, lines 10-12) to sequence the avermectin PKS, and acknowledge that "obvious ambiguities" in the sequence remain (page 126, legend of Figure 2). It is also of interest to note that the alleged sequence of the genes had not been deposited into a publicly available database to allow a skilled artisan to utilize the sequences to create hybrid PKSs. Therefore, the 'corresponding genes' are clearly not fully provided by MacNeil et al.

Applicants also submit that MacNeil et al. acknowledges on several occasions the difficulty that creating Type I hybrids would entail. Indeed, at page 130, MacNeil et al. teach that the "challenge to producing novel structures awaits further studies to determine rules for how domains can be inactivated, switched, or added to [a synthase unit] without inactivating the entire PKS protein." Clearly, MacNeil et al., at a time after the priority date of U.S. Patent No. 5,692,290, fail to provide a skilled artisan with the reasonable expectation of success that is necessary to sustain an obviousness rejection with respect to the instantly claimed invention.

To emphasize that the claimed invention overcomes the perceived difficulty in creating Type I/Type I hybrid PKSs that are not inactivated by the swapping of modules, Applicants have amended claims 52, 61-63, and 67 to recite that the hybrid PKSs are functional. Indeed, the instant specification provides methods for the generation of hybrid PKSs that are functional and produce the anticipated polyketide (see, e.g., the Examples such as Example 12). Specific support for this amendment can be found throughout the specification, including, at page 132, lines 10 through 13 and lines 24 through 27.

In light of the foregoing, Applicants respectfully request the withdrawal of this rejection of claims 2, 25, 26, 31-37, 39, 44, 47-49, 55-60, and 64-68 under U.S.C. §103.

CLAIM 27 IS NOT RENDERED OBVIOUS BY U.S. PATENT NO. 5,692,290 IN VIEW OF MACNEIL ET AL. AND KAO ET AL. AND CLAIMS 51 AND 52 ARE NOT RENDERED OBVIOUS BY U.S. PATENT NO. 5,692,290 IN VIEW OF MACNEIL ET AL. AND U.S. PATENT NO. 5,190,871

The Examiner has rejected claim 27 under 35 U.S.C. §103(a) as allegedly unpatentable over U.S. Patent No. 5,692,290 in view of MacNeil et al. and Kao et al. (Science

(1994) 265:509-512). The Examiner contends U.S. Patent No. 5,692,290 and MacNeil et al. teach that modular PKSs have "relaxed specificity for their starter units" and that Kao et al. specifically teach the relaxed specificity of the DEBS and avermectin PKSs.

The Examiner has also rejected claims 51 and 52 under 35 U.S.C. §103(a) as allegedly unpatentable over U.S. Patent No. 5,692,290 in view of MacNeil et al. U.S. Patent No. 5,190,871. It is the Examiner's position that U.S. Patent No. 5,190,871 teaches the stable integration of foreign DNA into a host cell's chromosomes and thereby renders obvious, in combination U.S. Patent No. 5,692,290 and MacNeil et al., the invention claimed in claims 51 and 52.

Applicants reiterate the arguments set forth above that U.S. Patent No. 5,692,290 and MacNeil et al. fail to render obvious the production of Type I-Type I hybrid PKSs which are functional to produce novel polyketides. Therefore, in the absence of an expectation of successfully producing Type I-Type I hybrid PKSs, Applicants submit that it would not have been obvious to arrive at the subject matter of claim 27 or claims 51 and 52, despite the teachings of Kao et al. and U.S. Patent No. 5,190,871, respectively.

Applicants add, however, that the knowledge that avermectin exhibits some flexibility for its starter unit does not provide a skilled artisan with a reasonable expectation of success that the incorporation of an avermectin loading module into a hybrid PKS would produce a functional enzyme. Indeed, in light of the caveats provided in Kao et al. and noted hereinabove (page 509, right column), a skilled artisan would not have predicted that the substitution of the avermectin loading module for the erythromycin loading module would have had the effect demonstrated in the instant application.

CONCLUSION

In view of the amendments presented herewith and the foregoing remarks, it is respectfully urged that the objections and rejections set forth in the August 21, 2003 Official Action be withdrawn and that this application be passed to issue.

In the event the Examiner is not persuaded as to the allowability of any claim, and it appears that any outstanding issue may be resolved through a telephone interview, the Examiner is requested to telephone the undersigned attorney at the phone number given below.

Respectfully submitted,

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Enclosures: Tanaka et al.
Figures 2B-19, 27, 29A-33

Energetic Predictions for Singlet and Triplet
atrienylidene[a]

set	wave function	total energy (hartrees)	rel energy (kcal/mol)
num	triplet SCF	−265.147 35	−15.3
num	singlet SCF	−265.110 89	7.6
num	singlet TCSCF	−265.122 94	0.0
	triplet SCF	−268.363 88	−4.7
	singlet SCF	−268.348 13	5.2
	singlet TCSCF	−268.356 34	0.0
-d	triplet SCF	−268.468 65	1.8
-d	singlet SCF	−268.458 80	8.0
-d	singlet TCSCF	−268.471 52	0.0

[a] ...al energy reported here corresponds to a completely op-...olecular structure of C_7H_6.

...[4] and of Kuzaj, Lüerssen, and Wentrup.[5]
...rlier molecular structure optimizations of RSV[1] could ...d in that they were carried out at the minimum basis ...) self-consistent-field (SCF) level of theory. In the ...search both structures were optimized by using a much ...uble ζ plus d function (DZ + d) basis set,[6] designated ...4s2p1d), H(4s/2s). The triplet state was described at ...-configuration SCF level of theory and the singlet state ...o-configuration (TC) SCF level of theory.[7]
...redicted structures of singlet and triplet cyclo-...ylidene are seen in Figures 1 and 2. Differences with ... the minimum basis (MBS) structures of RSV[1] are ...e but not qualitative in nature. For example, for the ...ucture, the DZ + d SCF C–C distances to the carbene ...e 1.427 Å, or 0.033 Å less than the earlier MBS SCF ... At an intermediate level of theory, DZ SCF for the ...e and DZ TCSCF for the singlet, we find in the present ... that both structures are predicted to be genuine ...that is, all 3 (13) − 6 = 33 vibrational frequencies are

...oretical energetic predictions are summarized in Table ... highest structurally optimized level of theory presented ...singlet energy falls below the triplet by 1.8 kcal/mol. ... the simplest carbene, the analogous DZ + d SCF/...vel of theory predicts a singlet–triplet separation of 12.3 ...le the experimental value (in accord with the highest ...theory) is ΔE(S–T) = 9.1 kcal.[9] If the analogy with ... is valid, one would expect the exact value of ΔE(S–T) ...ut 5 kcal for cycloheptatrienylidene.
...ove supposition is confirmed by higher level theoretical ...For example when the basis set for the carbene carbon ...ed to C(9s5p2d/7s4p2d), total energies of −268.477 47 ...TCSCF) and −268.471 90 hartrees (triplet SCF) are ...yielding a singlet–triplet splitting of 3.5 kcal/mol. Going ...he original DZ + d basis set, configuration interaction ...e functions including all single and double excitations ...determined. The corresponding energies are −269.174 65 ...wo-reference CISD) and −269.170 15 (triplet CISD), ...a singlet–triplet splitting of 2.8 kcal. Adding the two ...ns (1.7 kcal for basis set, 1.0 kcal for correlation effects) ...sults in Table I yields ΔE(S–T) = 4.5 kcal/mol.
...the clear theoretical prediction that cycloheptatrienylidene ...glet ground state, why is an EPR spectrum observed in ...atory? The simplest explanation would appear to be that ...*state is lower in energy than the singlet at the former's ...um geometry*. This hypothesis has been confirmed at ...d triplet SCF/singlet TCSCF level of theory. At the triplet equilibrium geometry, the singlet energy lies 6.7 kcal/mol higher. Thus, although the singlet state is indeed the true planar ground state (as predicted earlier[1]), the triplet state minimum is well separated geometrically (the singlet and triplet C–carbene C–C angles differ by 13.8°; analogous singlet and triplet bond distances differ by as much as 0.045 Å) and has a relatively long lifetime with respect to intersystem crossing.

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...inaga, S. *J. Chem. Phys.* **1965**, *42*, 1293. Dunning, T. H. *J. Chem.* ..., *53*, 2823.
...an early use and justification of this triplet SCF/singlet TCSCF ...see: O'Neil, S. V.; Schaefer, H. F.; Bender, C. F. *J. Chem. Phys.* ...162. For a more recent discussion in the context of the simplest ...carbene", see: Lee, T. J.; Bunge, A.; Schaefer, H. F. *J. Am. Chem.* ...*107*, 137.
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...aefer, H. F. *Science (Washington, DC)* **1986**, *231*, 1100.

Structure of FK506: A Novel Immunosuppressant Isolated from *Streptomyces*

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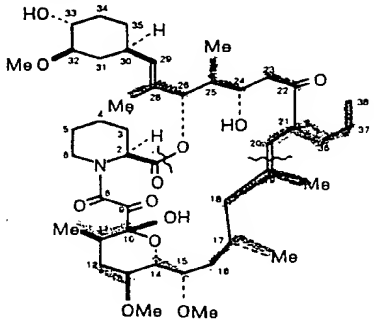
Considerable attention has recently been focused on the immunosuppressants represented by ciclosporins[1] because of their usefulness in bone marrow and organ transplantations. In the course of search for such immunosuppressive agents in our laboratories, FK506 (**1**), a novel 23-membered macrolide lactone, was isolated from *Streptomyces tsukubaensis* no. 9993. Herein we report the structural elucidation of this natural product.

FK506 (**1**) was isolated as colorless prisms from MeCN:[2] $C_{44}H_{69}NO_{12}$ (SIMS and elemental analysis[3]); mp 127–129 °C; $[\alpha]_D$ −84.4° (*c* 1.02, $CHCl_3$). The IR spectrum ($CHCl_3$) showed the presence of hydroxy groups (3700, 3600, 3550 cm^{-1}), carbonyl groups (1750, 1730, 1710 cm^{-1}), and an amide group (1650 cm^{-1}). The ^{13}C NMR spectrum ($CDCl_3$) revealed that **1** exists as an



1

equilibrium mixture of two isomers in solution (ca. 3:1 in $CDCl_3$). A detailed analysis of the spectrum[4] with the aid of the DEPT technique revealed all the carbon signals which are assignable to

(1) For a review on ciclosporins, see: *Ciclosporin, Progress in Allergy 38*; Borel, J. F., Ed.; Karger: New York, 1986.
(2) Kino, T.; Hatanaka, H.; Hashimoto, M.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.*, in press.
(3) SIMS, *m/z* 804 (M + 1); elemental analysis. Anal. Calcd for $C_{44}H_{69}NO_{12}\cdot H_2O$: C, 64.29; H, 8.71; N, 1.70. Found: C, 64.20; H, 8.86; N, 1.72.
(4) Spectral data for FK506 (**1**) and its degradation products (**2**, **3**, **6**, **7**, and **9**) are given in the Supplementary Material.

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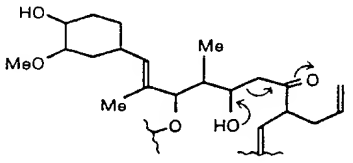
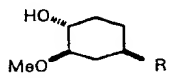
two ketones, one lactone (or ester), one amide, one vinyl, two trisubstituted olefins, one hemiketal (or ketal), three O-methyls, and five C-methyls, the remainder being 12 methylenes and 13 methines.

Partial structure A was suggested by the following experimental data. Ozonolysis of **1** (O_3/CH_2Cl_2, −78 °C) followed by reductive



A

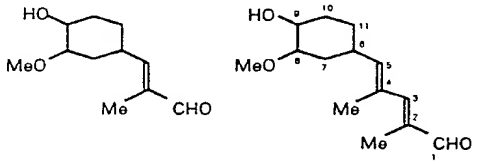


2 R=CHO

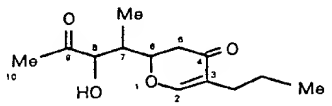
3 R=CH_2OH

and alkaline workup (1. Me_2S; 2. 1 N NaOH) gave aldehyde **2** (EIMS m/z 158 (M^+)).[4] Reduction of **2** with $NaBH_4$ (EtOH) gave the corresponding alcohol **3**, an inspection of whose ^1H NMR spectrum[4] with the aid of the COSY experiment presented the structure **3** for this compound and hence the structure **2** for the aldehyde. The structure of alcohol **3** was finally confirmed by identification with an authentic sample.[5]

Hydrolysis of **1** with 1 N NaOH (dioxane) produced α,β-unsaturated aldehyde **4** (EIMS m/z 198 (M^+): δ_H ($CDCl_3$) 1.78



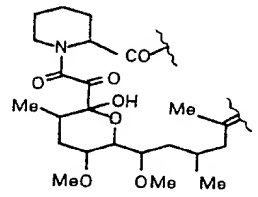
4 5



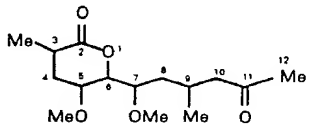
6

(d, J = 1.0 Hz, 3 H); δ_c ($CDCl_3$) 9.3 (q)), while treatment of **1** with NaH (THF, reflux) provided dienal **5** (EIMS m/z 238 (M^+): δ_H ($CDCl_3$) 2.00 (d, J = 1.3 Hz, 3 H), 1.95 (d, J = 1.1 Hz, 3 H); δ_c ($CDCl_3$) 16.2 (q), 10.7 (q)), establishing that two of the five Me groups in **1** are located at C-2 and C-4 in **5**.[6] Ozonolysis of the dihydro derivative of **1**, derived by catalytic reduction of the vinyl group in **1** (H_2 (1 equiv)/PtO_2/MeOH), gave, after reductive and alkaline workup, dihydropyrone **6** (EIMS m/z 24[illegible] (M^+)), whose structure was deduced on the basis of its spectroscopic data,[4] establishing the linkage of the propyl group (corresponding to the allyl group in **1**) to C-3 and the bonding of the hydroxy and dihydropyrone ring oxygens to C-8 and C-6, respectively, in **6**. The chemical evidence described above thus leads to the partial structure A.

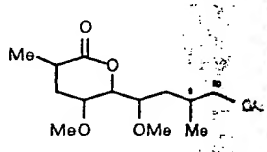
The partial structure B was derived on the following grounds. Acid hydrolysis of **1** (6 N HCl, reflux) gave L-pipecolic acid, which was identified by comparison with an authentic sample on HPLC.[7] The direct ozonolysis of **1** as described above also gave lactone **7** (SIMS m/z 287 (M + 1)), whose structure was assigned from



B



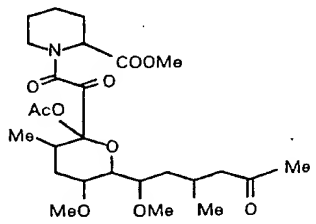
7



8

its NMR data including the COSY and C–H correlation experiments[4] except for the Me group tentatively assigned to be linked to C-9: the possibility remained that it is linked to C-10. Conversion of **7** to compound **8** (*m*CPBA/CH_2Cl_2), however, shifted the resonance of methylene protons (C-10) downfield to δ 4.00 (dd, J = 10.7, 5.6 Hz, 1 H) and 3.92 (dd, J = 10.7, 6.4 Hz, 1 H), confirming the validity of the structure **7** for the ozonolysis product.[8]

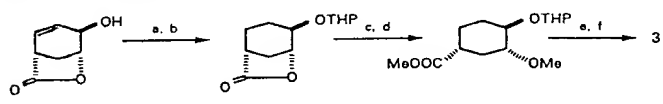
After alkaline treatment of **1** (1 N NaOH as described above) followed by methylation (CH_2N_2/Et_2O) and acetylation (Ac_2O/pyr), the reaction mixture was subjected to ozonolysis to give, after reductive workup, compound **9**, whose structural assignment was made by comparison of its ^{13}C NMR data[4] with those of **7** and methyl *N*-acetylpipecolate.[9] These chemical data thus postulate the partial structure B.



9

A reasonable connection of the partial structure A and B via lactone and olefin linkages (as shown by the wavy line in **1**) [illegible]

(5) Alcohol **3** was synthesized as follows. The starting material i was prepared according to the literature: Bartlett, P. A.; McQuaid, L. A. *J. Am. Chem. Soc.* **1984**, *106*, 7854.



i ii iii

[a] 2,3-DHP/PPTS/CH_2Cl_2. [b] H_2/10%Pd–C/EtOH. [c] MeONa/MeOH. [d] MeI/NaH/THF. [e] $LiAlH_4/Et_2O$. [f] HCl/MeOH.

(6) The formation of **4** or **5** can be rationalized by retroaldol cleavage, followed by further retroaldol or dehydration reactions.

(7) HPLC: column, CHIRALPAK WH (DAICEL CHEMICAL) [illegible] × 250 mm); eluent, 0.25 mM solution of $CuSO_4$ in H_2O; flow rate, [illegible] mL/min; temperature, 50 °C; retention time, 12.5 min (authentic sample L-pipecolic acid, 12.5 min; D-pipecolic acid, 9.1 min).

(8) The lactone carbonyl in **7** would be formed by an abnormal course oxidation of the masked α,β-diketo function. For similar abnormal oxidations, see, e.g.: Deslongchamps, P.; Moreau, C. *Can. J. Chem.* **1971**, [illegible] 2465.

(9) Methyl *dl*-*N*-acetylpipecolate was prepared from *dl*-pipecolic acid by esterification (MeOH/$SOCl_2$) followed by acetylation (Ac_2O/pyr); for the ^{13}C NMR data, see the Supplementary Material.

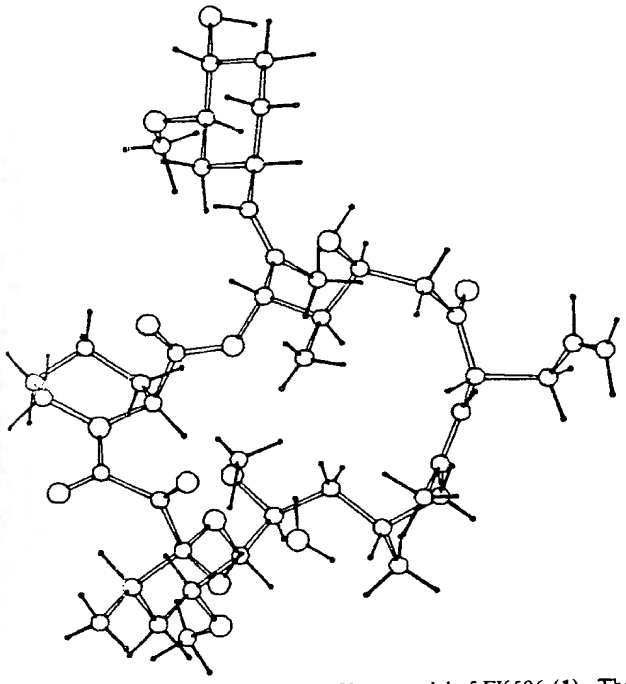


Figure 1. A perspective drawing of the X-ray model of FK506 (1). The water molecule is included.

the full structure of FK506. The geometry of the two trisubstituted olefins in 1 was assigned to both to be E on the basis of the upfield resonations of the Me groups bonded to these double bond (19-Me, δ_c 15.8; 27-Me, δ_c 13.9). Since several attempts to assign the stereochemistry of the other functional groups were unsuccessful, an X-ray analysis was performed on crystalline FK506 itself (Figure 1),[10] establishing the relative stereochemistry as depicted in 1. The absolute configuration was determined by the fact that 1 contains L-pipecolic acid (see above). The tautomeric equilibration of 1 in solution might be associated with a restricted rotation of the amide bond within the macrolide ring.[11,12]

FK506 represents a new class of macrolide lactones with amino acid and hemiketal-masked α,β-diketoamide functionalities incorporated in a 23-membered ring.[13] The activity of FK506 was considerably greater than that of cyclosporin A in various immunosuppression assays.[14]

(10) Crystal data for 1 ($C_{44}H_{69}NO_{12}\cdot H_2O$, M = 804.0): orthorhombic; space group $P2_12_12_1$; unit cell a = 10.939 (1) Å, b = 15.878 (1) Å, c = 27.184 (1) Å; V = 4721.0 Å^3; Z = 4; Dx = 1.131 g·cm^{-3}. Intensities were measured on a Rigaku AFC-5RU diffractometer by using graphite-monocromated Cu Kα radiation (λ = 1.5418 Å). Of 4484 independent reflections with $2\theta <$ 120°, 3249 were used for structure determination. The structure was determined by direct methods (RANTAN) and successive Fourier syntheses and block-diagonal least-squares. The final R factor, based on the used reflections, was 0.071.

(11) In comparison of the ^{13}C NMR signals of the major and minor isomers, the most significant differences in chemical shift were observed at C-2 and C-6. In the major isomer, C-2 resonated by 4.2 ppm to lower fields than in the minor isomer, while C-6 was observed by 4.7 ppm to upper fields, suggesting that in the major isomer the amide bond is in cis conformation in accord with the result of the X-ray crystal analysis.

(12) The ^{13}C NMR spectrum in solid state revealed that FK506 exists as the cis conformer (cis amide conformation): see the Supplementary Material.

(13) The closest literature analogue that contains these functionalities is chloramphenicin, which has been described as an antifungal antibiotic: Findlay, J. A.; Radics, L. *Can. J. Chem.* **1980**, *58*, 579.

(14) The exceptional activity of FK506 will be reported separately. (a) Kino, T.; Hatanaka, H.; Miyata, S.; Inamura, N.; Yajima, T.; Goto, T.; Kichara, M.; Kohsaka, M.; Aoki, H.; Ochiai, T. *J. Antibiot.*, in press. (b) Inamura, N.; Nakahara, K.; Kino, T.; Goto, T.; Aoki, H.; Yamaguchi, I.; Kosaka, M.; Ochiai, T. *Transplantation*, in press. (c) Ochiai, T.; Nakajima, K.; Nagata, M.; Hori, S.; Asano, T.; Isono, K. *Transplantation*, in press. (d) Ochiai, T.; Nagata, M.; Nakajima, K.; Suzuki, T.; Sakamoto, K.; Enomoto, K.; Gunji, Y.; Uematsu, T.; Goto, T.; Hori, S.; Kenmochi, T.; Nakagouri, T.; Asano, T.; Isono, K.; Hamaguchi, K.; Tsuchida, H.; Nakahara, K.; Inamura, N.; Goto, T. *Transplantation*, in press.

Supplementary Material Available: Spectral data (IR, ^{1}H NMR, and ^{13}C NMR) for **1**, **2**, **3**, **6**, **7**, and **9** and additional X-ray crystallographic data for **1** (9 pages). Ordering information is given on any current masthead page.

Molecular Recognition: Hydrogen Bonding and Stacking Interactions Stabilize a Model for Nucleic Acid Structure

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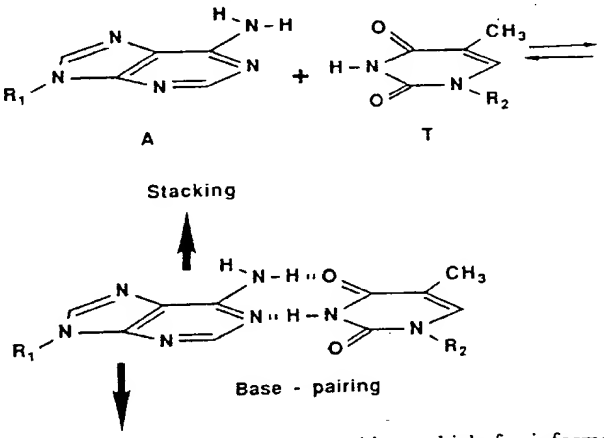
The classical form of molecular recognition is the base pairing within nucleic acids as formulated by Watson and Crick.[1] The complementary hydrogen bonding surfaces shown in eq 1 for



(1)

adenine (A) and thymine (T) provide a vehicle for information transfer, while stacking interactions between adjacent base pairs provide additional stability for the helical structure.[2] The hydrogen bonding aspects of eq 1 have been examined in detail by Rich[3] and Hammes[4] with use of derivatives of A and T in the noncompeting solvent $CDCl_3$, while the stacking of individual bases in H_2O was observed by Chan.[5] Here, we introduce a model system in which both forces can operate simultaneously.

The new models, e.g., **1**, are designed in accord with the principles of molecular recognition[6] and feature stacking and hydrogen bonding surfaces that converge on the substrate from perpendicular directions. Moreover, their bulk reduces the dimerization (self-recognition) that is generally observed[3] in addition to eq 1. The scaffolding for the new structures is provided by derivatives of Kemp's triacid[7] **2**, in which the U-shaped relationship that exists between any two carboxyl functions is enforced by the equatorial methyl groups. Sublimation of **2** or its successive treatment with $(CF_3CO)_2O$ and water gives the anhydride acid[7] **3a**. With NH_4OH **3a** gives the imide acid[8] **3b** (mp > 280 °C) from which the acid chloride **3c** (mp 171 °C) can be obtained with $SOCl_2$. The new amides are obtained by acylation of the aromatic amines **4a–e** with **3c**. In addition, the methyl ester **3d**

(1) Watson, J. D.; Crick, F. H. C. *Nature (london)* **1953**, *171*, 737–8.
(2) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; Chapter 6.
(3) Kyogoku, Y.; Lord, R. G.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 250–257. Iwahaski, H.; Kyogoku, Y. *J. Am. Chem. Soc.* **1977**, *99*, 7765.
(4) Hammes, G. C.; Park, A. C. *J. Am. Chem. Soc.* **1968**, *90*, 4151–57.
(5) Chan, S. I.f Schweitzer, M. P.; Ts'o, P. O. P.; Helmkamp, G. K. *J. Am. Chem. Soc.* **1964**, *86*, 4182. Schweitzer, M. P.; Chan, S. I.; Ts'o, P. O. P. *J. Am. Chem. Soc.* **1965**, *87*, 5241.
(6) Rebek, J., Jr. *Science (Washington, DC)* **1987**, *235*, 1478–1484.
(7) Kemp, D. S.; Petrakis, K. S. *J. Org. Chem.* **1981**, *46*, 5140–43.
(8) All new compounds were characterized by 300-MHz PMR, 75-MHz ^{13}C NMR, and FTIR spectroscopy. Elemental analyses were either within 0.3% of calculated combustion values or within 0.001 of calculated mass spectral values.

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